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Hepatitis C Virus Late Relapse after Sustained Virologic Response from Interferon and Ribavirin Treatment as Confirmed by RNA Sequencing

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Hepatitis C virus (HCV) viremia is unusual (<5%) after successful treatment, defined as sustained virologic response (SVR) or undetectable HCV PCR 12 to 24 weeks after therapy. We present a case of late virologic relapse (*de novo* infection was excluded by RNA sequencing) after SVR followed by spontaneous viral clearance.

CASE REPORT

A 49-year-old woman without significant past medical history was evaluated for hepatitis C virus (HCV) genotype 3a cirrhosis. Her only identified risk factor was intravenous gamma globulin transfusion in 1972. Her presenting HCV load was 2.64×10^5 IU/ml. Her serum alanine aminotransferase (ALT) level was 82 U/liter, aspartate aminotransferase (AST) was 113 U/liter, albumin was 25 g/liter, bilirubin was 26 μmol/liter, and international normalized ratio (INR) was 1.3, and her platelets were 49×10^9/liter. Initial HCV treatments using weight-based dosing failed twice due to relapse, defined as HCV PCR being negative on treatment but then positive after stopping treatment. Treatment 1 was administered in January 2001 with standard alpha interferon (IFN-α2b) at 3 MU three times per week and ribavirin (RBV) at 1,200 mg daily for 48 weeks. Treatment 2 was administered in March 2003 with pegylated (PEG) IFN-α2b at 120 μg weekly and RBV at 1,000 mg od for 24 weeks. Both treatments were complicated by neutropenia, leading to dose reductions. Treatment 3 was administered in November 2006 with PEG IFN-α2b at 100 μg weekly and RBV at 1,200 mg daily for 22 weeks. Treatment was stopped early, with no dose reduction on therapy, due to an episode of spontaneous bacterial peritonitis. Nonetheless, the patient achieved a sustained virologic response (SVR) with negative HCV PCR results in November 2007, 29 weeks posttreatment, with normalization of ALT.

It was not routine clinical practice to repeat HCV PCR testing after SVR. However, the ALT level became abnormal again, peaking at 109 U/liter (Fig. 1) in February 2009. HCV PCR testing was repeated then and confirmed HCV infection with genotype 3a. A detailed history revealed no risk factors for reinfection. HCV sequencing performed on stored sera from November 2006 (pretreatment) and December 2009 (posttreatment) confirmed relapse after SVR rather than new infection. The clinical course was reminiscent of acute infection, with wildly fluctuating viral loads, followed by spontaneous resolution without antiviral therapy.

HCV RNA was extracted from 250 μl of pre- and posttreatment sera using a NucliSENS easyMag automated nucleic acid extraction system (bioMérieux Inc., Durham, NC) and was amplified by reverse transcription-PCR using primers specific for the 5′ noncoding region and the Core, E1, and NS5B genes. Sequence data obtained were used to determine the HCV genotype and phylogenetic relatedness. Genetic distances were estimated by

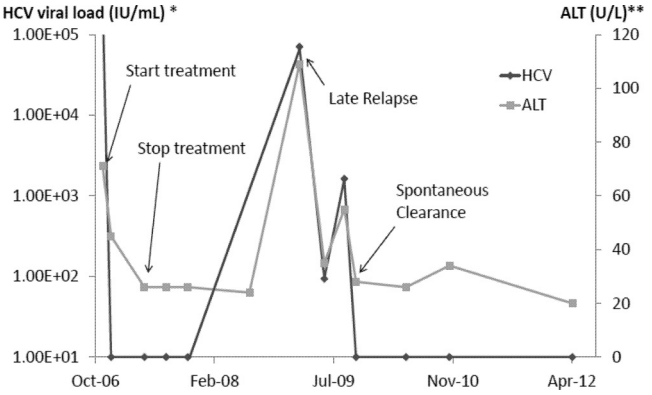

FIG 1 Evolution of serum HCV RNA load through time. After HCV treatment from November 2006 to April 2007, SVR was followed by a late relapse in February 2009 and spontaneous clearance in October 2009. *, lower limit of HCV RNA detection = 15 IU/ml; **, normal ALT range ≤ 40 U/liter.

Kimura two-parameter analysis, and a phylogenetic tree was constructed by the maximum-likelihood method. Significant taxonomic relationships were obtained by bootstrap resampling analysis (200 replicates) (confidence values of 70% or greater are considered significant).

A phylogenetic tree of 50 HCV strains (Fig. 2) was prepared to determine genetic relatedness. Forty-two randomly selected HCV subgenotype 3a strains from three Canadian provinces were aligned with four strains belonging to the same subgenotype as that collected from our patient. To better illustrate the difference between reinfection and relapse after SVR, three additional HCV strains from a patient with reinfection (initially suspected to represent a late relapse) were also included. A HCV subgenotype 1a sequence was used as an outlier.

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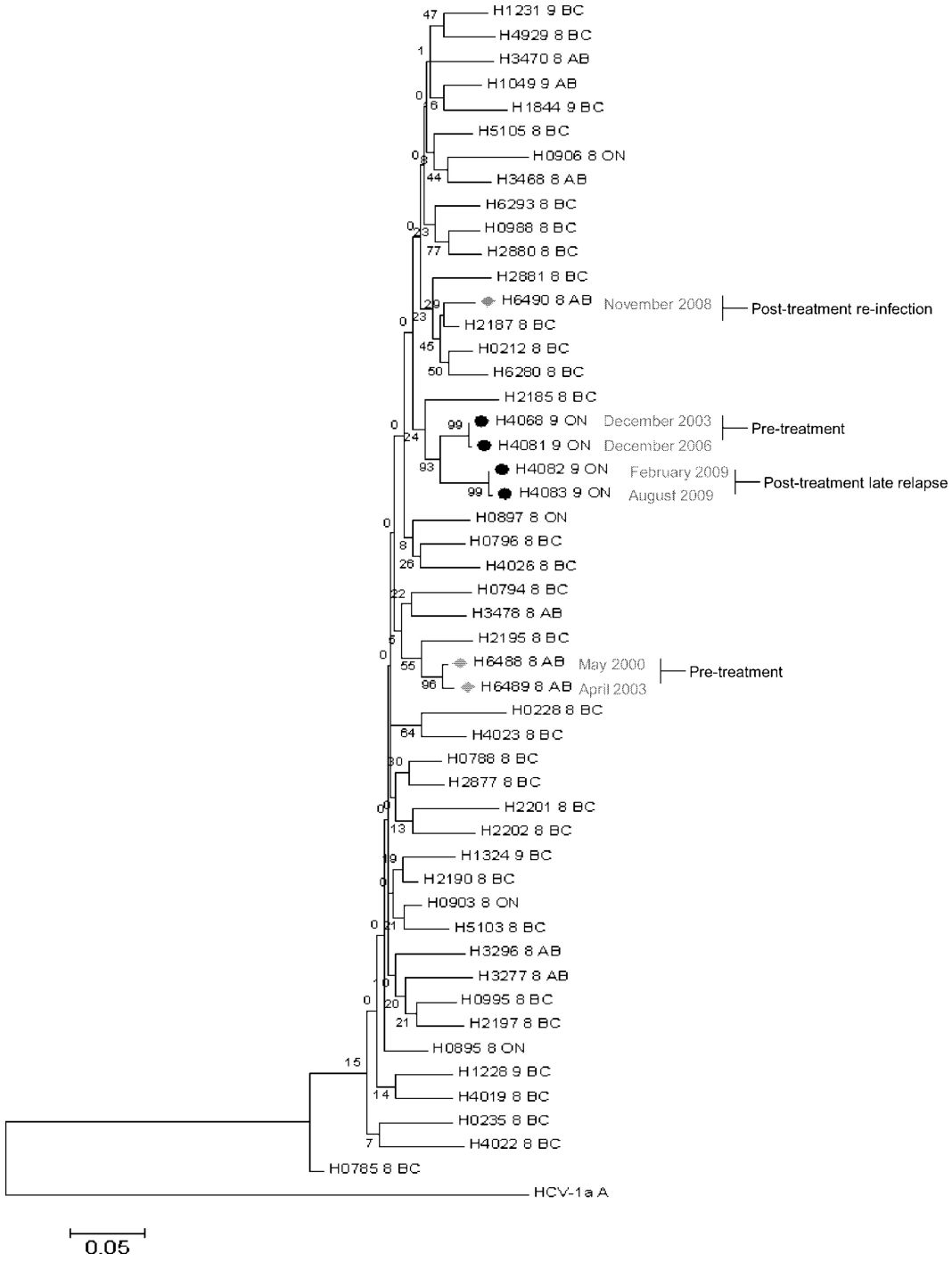

FIG 2 Phylogenetic analysis of 50 HCV E1 coding region sequences. The phylogenetic tree was constructed using sequences from 50 HCV genotype 3a strains and one sequence from a genotype 1a strain used as an outlier. Each sequence is denoted by a code number and a single digit indicating the year (8, 2008; 9, 2009). Ovals designate pretreatment samples (H4068 collected in 2003 and H4081 in 2006) that were closely related to probable late-relapse samples (H4082 and H4083 collected in 2009). For contrast, diamonds designate pretreatment samples (H6488 collected in 2000 and H6489 in 2003) that were not related to sample H6490 collected in 2008, indicating a reinfection rather than relapse. AB = Alberta; ON = Ontario; BC = British Columbia.

Older sequences from samples collected from our patient before treatment (2003 and 2006) were very similar (99% sequence homology). The ones obtained after the relapse post-SVR (February and August 2009) exhibited even higher sequence homology—99.8% (Fig. 2). The same phylogenetic relatedness between the HCV strains was observed with sequences from the Core and NS5B genes (data not shown). All four sequences clustered together by branching on the same

subnode. Relatedness is supported by a very high bootstrap value (node bootstrap of 93) (Fig. 2). That well-defined cluster is located apart from the rest of the HCV subgenotype 3a isolates.

In contrast, there was no phylogenetic relatedness between the pretreatment HCV isolates from the 37-year-old male patient with HCV genotype 3a included in Fig. 2 and his 2008 HCV isolate, indicating *de novo* infection. It is of interest that the viral strains from these two patients were quite stable, with minimal change during the last 3 years before treatment based on the sequence homology mentioned above. This is an important consideration when analyzing the phylogenetic relatedness of different HCV strains because the virus/host interaction in some cases speeds up the mutation rate, leading to increased variability of the virus genome, which makes it difficult to assess phylogenetic relationships in the absence of intermediary samples collected throughout the years.

SVR is accepted as a durable treatment endpoint in HCV infections (1, 2). Cases of serum HCV RNA detected after SVR have been reported (3–8). However, most studies do not distinguish between relapse after SVR and *de novo* reinfection. Detection of serum HCV RNA should further be differentiated from the more frequently documented—yet of uncertain clinical significance—"occult HCV," where HCV RNA is undetectable in serum but detectable in hepatocytes or peripheral blood mononuclear cells (7).

Veerapu et al. report a series of 98 patients treated with IFN and RBV, where 15% of patients had detectable serum HCV RNA after SVR (9). In all cases, with one exception, RNA was detectable only when using nested PCR rather than conventional PCR. Moreover, the authors analyzed the highly conserved 5′ untranslated region, which is less sensitive for distinguishing relapse from *de novo* infection within the same genotype subtype.

We found two cases of late relapse confirmed through sequencing of high-variability regions: one in a patient with hypogammaglobulinemia after IFN treatment (10) and the other after IFN and RBV treatment in which late relapse progressed to chronic infection (11).

This case is unique in that we were able not only to confirm relapse after SVR following IFN and RBV treatment but also to document spontaneous resolution. It is furthermore important to acknowledge the complex interplay between several factors such as genotype, age, and duration of therapy that influenced response to treatment in this patient, leading to HCV clearance (12–14). In this individual, SVR represented HCV suppression rather than HCV eradication. Furthermore, relapse was asymptomatic and was detected only after investigation of newly elevated transaminase levels. The possibility that viremia after SVR occurred before the rise in the ALT level cannot be eliminated. It is possible that late events might happen more frequently but without being recognized. We speculate that SVR achieved using new IFN-free regimens may be more durable due to viral eradication rather than immune suppression.

SVR is a durable and clinically significant endpoint of interferon-based HCV therapy. Relapse after SVR is rare but potentially unrecognized as it is likely clinically silent, and viremia can be self-limited.

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REFERENCES

1. **Formann E, Steindl-Munda P, Hofer H, Jessner W, Bergholz U, Gurguta C, Ferenci P.** 2006. Long-term follow-up of chronic hepatitis C patients with sustained virological response to various forms of interferon-based anti-viral therapy. Aliment. Pharmacol. Ther. **23:**507–511. http://dx.doi.org/10.1111/j.1365-2036.2006.02785.x.
2. **Maylin S, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, Giuily N, Castelnau C, Cardoso AC, Asselah T, Feray C, Nicolas-Chanoine MH, Bedossa P, Marcellin P.** 2008. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. Gastroenterology **135:**821–829. http://dx.doi.org/10.1053/j.gastro.2008.05.044.
3. **Veldt BJ, Saracco G, Boyer N, Camma C, Bellobuono A, Hopf U, Castillo I, Weiland O, Nevens F, Hansen BE, Schalm SW.** 2004. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. Gut **53:**1504–1508. http://dx.doi.org/10.1136/gut.2003.038257.
4. **Desmond CP, Roberts SK, Dudley F, Mitchell J, Day C, Nguyen S, Pianko S.** 2006. Sustained virological response rates and durability of the response to interferon-based therapies in hepatitis C patients treated in the clinical setting. J. Viral Hepat. **13:**311–315. http://dx.doi.org/10.1111/j.1365-2893.2005.00685.x.
5. **Giannini EG, Basso M, Savarino V, Picciotto A.** 2010. Sustained virological response to pegylated interferon and ribavirin is maintained during long-term follow-up of chronic hepatitis C patients. Aliment. Pharmacol. Ther. **31:**502–508. http://dx.doi.org/10.1111/j.1365-2036.2009.04201.x.
6. **Swain MG, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, Abergel A, Pessoa MG, Lin A, Tietz A, Connell EV, Diago M.** 2010. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. Gastroenterology **139:**1593–1601. http://dx.doi.org/10.1053/j.gastro.2010.07.009.
7. **Welker MW, Zeuzem S.** 2009. Occult hepatitis C: how convincing are the current data? Hepatology **49:**665–675. http://dx.doi.org/10.1002/hep.22706.
8. **Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, Wilkinson J, Adair D, Rakela J, Laskus T.** 2005. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. Hepatology **41:**106–114. http://dx.doi.org/10.1002/hep.20518.
9. **Veerapu NS, Raghuraman S, Liang TJ, Heller T, Rehermann B.** 2011. Sporadic reappearance of minute amounts of hepatitis C virus RNA after successful therapy stimulates cellular immune responses. Gastroenterology **140:**676–685.e1. http://dx.doi.org/10.1053/j.gastro.2010.10.048.
10. **Lee WM, Polson JE, Carney DS, Sahin B, Gale M, Jr.** 2005. Reemergence of hepatitis C virus after 8.5 years in a patient with hypogammaglobulinemia: evidence for an occult viral reservoir. J. Infect. Dis. **192:**1088–1092. http://dx.doi.org/10.1086/432917.
11. **Fujii H, Itoh Y, Ohnishi N, Sakamoto M, Ohkawara T, Sawa Y, Nishida K, Nishimura T, Yamaguchi K, Yasui K, Minami M, Okanoue T, Ohkawara Y, Yoshikawa T.** 2010. Relapse of hepatitis C in a pegylated-interferon-alpha-2b plus ribavirin-treated sustained virological responder. Hepatol. Res. **40:**654–660. http://dx.doi.org/10.1111/j.1872-034X.2010.00641.x.
12. **Brjalin V, Salupere R, Tallo T, Kuznetsova T, Priimagi L, Tefanova V.** 2012. Efficacy of peginterferon alpha-2A and ribavirin combination therapy in treatment-naive Estonian patients with chronic hepatitis C. Cent. Eur. J. Public Health **20:**150–155.
13. **Oze T, Hiramatsu N, Mita E, Akuta N, Sakamoto N, Nagano H, Itoh Y, Kaneko S, Izumi N, Nomura H, Hayashi N, Takehara T.** 2013. A multicenter survey of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan. Hepatol. Res. **43:**35–43. http://dx.doi.org/10.1111/j.1872-034X.2012.01056.x.
14. **Inglot M, Pawlowski T, Szymczak A, Malyszczak K, Zalewska M, Radkowski M.** 2013. Replication of hepatitis C virus in peripheral blood mononuclear cells in patients with chronic hepatitis C treated with pegylated interferon alpha and ribavirin. Postepy. Hig. Med. Dosw. (Online) **67:**186–191. http://dx.doi.org/10.5604/17322693.1038785.